

ON THE INHERITANCE OF BODY FORM AND OF CERTAIN
OTHER CHARACTERISTICS, IN THE CONJUGATION
OF EUPLOTES PATELLA

BERNARD M. COHEN

A DISSERTATION

submitted to the Board of University Studies of the
Johns Hopkins University in conformity with
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INTRODUCTION

The present paper is an account of results obtained from conjugations within a clone of *Euplotes patella* of atypical form, and within clones descended from some of the ex-conjugants produced. In the foregoing paper (COHEN 1934) on the effects of conjugation in this hypotrichous ciliate a study was made of a group of conjugants obtained by inducing conjugation among the members of a single clone of typical elliptical form. The two members of one pair of conjugants, pair $\beta 51b$, were found after separating to have become altered from the elliptical to a circular form. This form was assumed, as far as could be detected, as soon as the animals had completed the changes of the reconstruction stage which occurs at conjugation. (See the foregoing paper for an account of these changes during the reconstruction stage.) Camera lucida drawings of a "circular" and a typical elliptical animal are reproduced here in figure 1 to show the difference in form between the two.

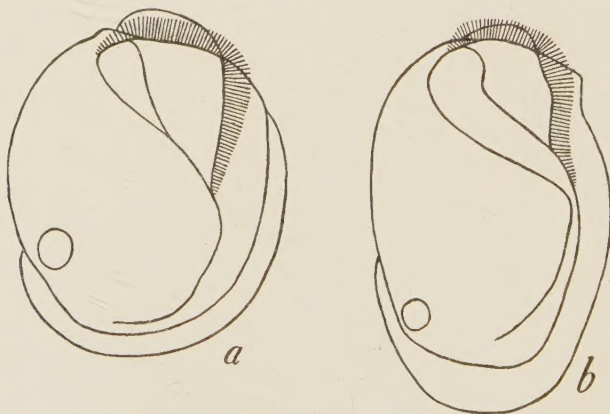


FIGURE 1.—Camera lucida drawings of a circular adult, a, and an elliptical adult, b. Iodin fixation.

The essential facts about the origin, early treatment, permanence, and characteristics of the structurally peculiar individuals may be summarized as follows:

1. The new form appeared in only 2 ex-conjugants (one pair) out of 271. It was recognized in each only after the two had been isolated in separate moist chambers.

2. The low frequency seems to indicate a mutation coincident with conjugation rather than the selecting out of already existing factors. However, a frequency of this order could result from a dependence of the characteristic on multiple factors, and since in the present case the term "mutation" has no further factual meaning than simply "low frequency," it will probably be best to regard the appearance of the new characteristic only as a result of conjugation.

3. The peculiarity was inherited consistently through vegetative reproduction during 30 days of observation of the isolated lines. The two lines derived from the two members of the pair were in different moist chambers throughout this time and were handled, and their records made, independently of each other.

4. In addition to the diversity in form, the individuals of the two lines differed also from the normal elliptical in their gross internal structure. The difference consisted essentially of the slight distortion of a clearly delimited area which appears opaque with transmitted light. This distortion may be characterized as what would occur if an elliptical animal were slightly compressed mechanically in an antero-posterior direction.

5. The fission rates of the two circular lines were very similar and unusually low. For one the mean daily rate was .70 fissions per day and for the other .73 fissions per day. Out of the total of 175 lines whose fission rates were determined, only three had rates lower than these two, and since the mean of the group was 1.12 fissions per day, these two were rather extreme variants. Considering the independence in culturing, the similarity in fission rate must have been due to genetic likeness.

6. Mass cultures of the two races, started with individuals from the isolation lines, were maintained as pure clones by daily attention. These showed no reversions to the elliptical type.

The circular form in this species has previously been found in nature. SAVILLE KENT (1882), in his description of *Euplotes patella*, says (p. 798): "This species, like many other Hypotricha, is subject to considerable local and individual variation, chiefly evinced in this instance in the varying contour of the carapace, and in the character of its ornamentation. This carapace or cuirass, while normally oval or elliptical, may be nearly circular or lozenge-shaped, broad in the center and much narrower at the antero-posterior extremities." From such an account, of course, it cannot be learned if such variants are distinct clones, as is true in the present case, but it is possible that they are, and that we have in the present case a

direct observation on the probable means by which some of the diversities found in nature come to exist, namely, as a result of conjugation.

No record has been found of any other case essentially similar to the one under consideration here. The unique features of this characteristic are its origin during conjugation, its affecting the descendants of both conjugants, its persistence through vegetative reproduction, and as will be shown later, its mode of inheritance through subsequent conjugations. Morphological diversities that persist through vegetative reproduction have been demonstrated before in protozoa, but with no great frequency considering the amount of work that has been done on the genetics of these forms. They have been described by JENNINGS (1913) and STOCKING (1915) in *Paramecium caudatum*, by DAWSON (1926) and MOORE (1927) in *Paramecium aurelia*, by REYNOLDS (1923) and JOLLOS (1924) in the rhizopod *Arcella*, and by MACDOUGALL (1931, 1931a) in *Chilodon*.

However, even in these comparatively few descriptions, it is only in respect to the one feature of permanence of the visible character through successive fissions that all of them are similar to what has been observed in *Euplotes*. In regard to the origin of the diversities, in only three accounts is this known at all; in two of these it appears that the characteristic arose as in the present case during conjugation. This last is shown by JENNINGS and by STOCKING in *Paramecium caudatum*. JENNINGS observed that after conjugation the lines from some ex-conjugants showed a persistent tendency to produce abnormal individuals of indefinite and usually bizarre shape. This was evidenced with varying frequency in diverse lines. STOCKING made an intensive study of this, working out in detail and establishing the conclusions of JENNINGS, that as a result of conjugation some lines acquired a genetically permanent tendency to become abnormal and this was expressed in different degrees in various lines. However, while such abnormalities are indeed structural, they differ from the peculiarity in *Euplotes* in their inconstancy of manifestation.

The third case in which the origin of the diversity is known is MACDOUGALL'S recent work on the production of a structural peculiarity by ultra-violet radiation in *Chilodon uncinatus*. Normal specimens of *Chilodon*, when radiated, were found to give rise to mutated forms which, in addition to less conspicuous structural changes, possessed a clearly discernable posterior prolongation, which she calls a "tail." It was shown in this work that inbreeding of the tailed forms resulted in the production anew of both normal and tailed individuals. In addition the other less conspicuous characters, which consist of a new form and new ciliation, may in some inbreedings be segregated from "tailed" and thereafter be inherited independently. In other inbreedings these characters are not separated but remain together consistently. MACDOUGALL has also twice

succeeded in crossing diverse clones of *Chilodon*. In one such cross-conjugation between a homozygous tailed clone and a normal clone none of the conjugants survived. Another between the heterozygous tailed form and the normal was successful but the results have not yet been published.

Besides this work of MACDOUGALL only one other paper, that of DAWSON, discusses the behavior of the structural diversity in conjugation. DAWSON found that the character he studied persisted after the animals possessing it had conjugated together.

The explanation of such phenomena as those observed in *Chilodon* and, as will be shown, in *Euplotes*, seems to involve the kind of genetic mechanism that has been so extensively demonstrated in the metazoa. It is known that in many species of Protozoa nuclear processes occur at conjugation that are in general similar to those involved in the formation of gametes and zygotes in higher forms. There is likewise reason to believe that protozoan chromosomes, at least in some genera of ciliates, are not essentially different in structure or behavior from those of the well-studied higher forms. As far as behavior is concerned, there is much evidence that they are similar. In a recent work by TURNER (1930) on the cytology of division and conjugation in *Euplotes patella*, in which he describes the chromosomes of this species, he shows the diploid number to be eight, and in pronucleus formation at conjugation, he finds that these are reduced quite conventionally to four. Subsequently, after the exchange of pronuclei between the members of the conjugating pair, fusion of the migratory and stationary pronuclei occurs in each with restoration of the chromosome number to eight. Similar meiotic phenomena involving definite chromosomes have been described in many other protozoa. However, no exact relation has as yet been demonstrated between the chromosomal behavior in protozoa and what is known of their genetics. A suggestive approach has been made by PASCHER who described (1916, 1918) the production of clear cut intermediate types by crossing two different species of the flagellate *Chlamydomonas*. Union of two gametes, one from each of the species, resulted in a cyst which was intermediate in form between the cysts of the two species formed by inbreeding. In *Chlamydomonas* swarm spores are produced in the cyst by fissions in which chromosome reduction occurs. When formed from the hybrid cysts, these swarm spores were found in some cases to be again like those of the parents, in other cases to consist of four types, having four diverse combinations of the characteristics of the parents.

The present investigation was carried on under the direction of Professor H. S. JENNINGS, for whose interest in the work and generous assistance in the preparation of this paper the author wishes to record his appreci-

individual isolated August 6, 1929. The conjugation in which the circulars were produced occurred on November 29, 1929; and on December 4 the clone which was used in the present work was started from an isolation line of the experiment in progress at that time.

The first group of conjugants to be examined, B1, arose March 30, 1930 from clone 51b. Of these, 48 pairs were taken for study. In addition to this the clone was maintained as such by continuing a culture of non-conjugants, and on April 8, as shown in the figure, it yielded another group of ex-conjugants, B2, of which 72 pairs were studied. In the meantime clones derived from four ex-conjugants of group B1, ex-conjugants 1b, 6a, 8b, and 45a, had been isolated as mass cultures from the respective isolation lines of that group and each of these yielded new "F2" groups of conjugants which were subsequently studied. From clone 6a arose on March 6 group C, of which 72 pairs were isolated for study; from clone 45a arose on April 1 group D1 and on April 9 group D2, in each of which 72 pairs were studied; clone 8b yielded group E on May 17 and clone 1b group F on May 21, from each of which 48 pairs were taken. The detailed treatment of each of these various groups and the experimental data obtained will be discussed in the next section. In following that discussion, reference to figure 2 will aid in making clear the derivations of the different groups and their relation to each other.

EXPERIMENTAL RESULTS

The Inheritance of Form

a. Group B1

As stated above, this group comprised 48 pairs that were isolated on March 3, 1930 from a culture of the circular clone 51b that was found conjugating on that day. Of these, four pairs had not separated by the following day so that the two members of but 44 pairs could be isolated. Of the four pairs which remained attached, separation later failed to occur, and they died in this condition within a few days. One member of one of these pairs, however, before dying and while attached to its mate, gave rise by fission to an individual which continued to live and reproduce. (This individual, which received the designation 45a and is seen thus to be the progenitor of one of the clones in which conjugation was studied later, is not of particular interest here aside from its being derived from an attached pair. In this respect it differs from the other ex-conjugants. It will be discussed in detail later in the accounts of groups D1 and D2.) Of the 89 animals which were thus obtained, 54 failed to develop from the dedifferentiated stage which normally follows conjugation, and died in this condition within six to nine days. The others were all seen to have rediffer-

entiated by the fourth day following conjugation (March 7) and on the following day most had begun to divide.

On the twenty-ninth day after conjugation all but two of the isolation lines were found dead. This wholesale mortality was clearly accidental since mass cultures previously started from some of the lines that died continued to live in good condition. The cause could not be determined. However, the data acquired showed some interesting indications.

The fact of greatest interest that appeared was that conjugation in this circular clone resulted in the production of three types of morphologically diverse lines: typical ellipticals, circulars, and intermediates. Specifically, of the lines derived from the 35 ex-conjugants which became redifferentiated:

- 15 were elliptical
- 12 were circular
- 8 were intermediate

This classification was made on the basis of the daily observations described in the preceding section. As shown by the actual records, the elliptical class included only lines that were distinctly and consistently elliptical, and the circular class only those which were distinctly and consistently circular; as such, both were clear-cut. The intermediate class, however, includes mainly lines whose form on most days was doubtful, in some lines being not clearly elliptical, in others not clearly circular. However, a few lines of this class showed consistently a form intermediate between elliptical and circular. But the frequent difficulty of distinguishing between these different types of intermediates forced the inclusion of all of them into a single class. The terms elliptical, circular, and intermediate will be used hereafter precisely as here defined. Of the 35 lines, 14 became thin and weak and died out before the later accidental deaths of the others. They included lines of each type. This fact will be discussed in the section on the relation of fission rate to form.

b. Group B2

On April 8, 1930, a mass culture of clone 51b was again found conjugating. This time 72 pairs were isolated to form group B2. On the following day the members of all pairs had separated, but they were not isolated at once. On April 12, four days after conjugation, 68 of the 144 individuals had become redifferentiated, and only these were transferred to fresh slides. The others were retained, however, and their deaths as still undifferentiated individuals was subsequently witnessed. The 68 fully redifferentiated animals were allowed to proliferate on the slides for four days without transferring, with the addition of fresh fluid at intervals. On the fourth

day, April 16, the forms of the animals of each line were recorded, and two to six, in most cases four, individuals were transferred to a fresh slide. This procedure was repeated two days later, on April 18, and four days later, on April 20. In this way three separate recordings of the forms of the different lines were obtained.

Apparently because of the unsatisfactory features of this type of treatment, although the specific reasons are unknown, 33 of the lines were weakened by the time the observations were made, to the extent that their form could not be determined. During the course of the observations 7 of these died. Of the remaining 35:

16 were elliptical

10 were circular

9 were intermediate

In addition to these two "F1" groups, two "F2" progeny groups, E and F, furnished data on the inheritance of form. E was derived from the circular clone 8b, and F from the circular clone 1b, both of which were descended from ex-conjugants of group B1. The remaining F2 groups, C, D1, and D2, did not provide data for this particular question.

c. Group E

Clone 8b was found conjugating on May 17 and 48 pairs were isolated on slides. All had separated by the following day, and on May 21, four days after conjugation, the redifferentiated individuals, consisting of 18 pairs and 12 singles whose mates had died, 48 animals in all, were transferred to fresh slides. These were then allowed to multiply on the slides for five days, and during this time they were observed daily and several times a drop of fresh culture fluid was added to each concavity. At the end of this period, on May 26, the form of the animals of each line was observed and recorded. As in the previous experiments no appreciable variation in form was seen among the individuals of any one line. A representative of each living line was then transferred to a fresh slide and after two days the form of the individuals of each line was again recorded. In those cases in which the records agreed perfectly as to whether the individuals of a line were clearly elliptical or clearly circular the line was classified accordingly. In a few cases where, in the first recording the form was uncertain while in the second it was clearly circular, these lines were classed as circular. All others which were uncertain in either record were classed as intermediate. There were four lines whose bad condition, resulting in a thin, pale and weak appearance, did not permit classification. Of the 44 remaining:

15 were elliptical

16 were circular

13 were intermediate

d. Group F

On May 21 clone 1b was found conjugating and 48 pairs were isolated. This group received the same treatment as group E, except that most of the redifferentiated individuals, of which there were 43, were put on fresh slides, separating the members of pairs, late on the third day following conjugation when redifferentiation could first be clearly detected. The remaining redifferentiated animals were not removed to fresh slides until the fifth day. (Their development was not certain until the fourth day). The lines of F were allowed to multiply for four days without transferring, with daily observation and addition of fresh culture fluid at intervals. The day of the first recording for group F, May 28, was the same as that of the second recording of group E. The second recording of F was on May 30. In the latter group 15 lines, as compared with 4 of E, did not permit of classification because of their weak appearance. Of the remaining 28 lines:

11 were elliptical
8 were circular
9 were intermediate

e. Summary: a-d

The results so far discussed are summarized in table 1 which gives also the percentage in each class of the total number of lines classified in each group. These percentages give some suggestion of uniformity of ratio in the production of each type of form. The possibility of interpreting the results in terms of Mendelian factors at once suggests itself, but this will be left to the concluding discussion, after all relevant data have been presented.

TABLE 1

Number of lines in each class and their percentage of the total number classified, in each group, in the total for four groups, in the two F1 groups, and in the two F2 groups.

GROUP	GENETIC RELATIONSHIP	NUMBER LINES CLASSIFIED	ELLIPTICAL		CIRCULAR		INTERMEDIATE	
			NUMBER	PERCENT	NUMBER	PERCENT	NUMBER	PERCENT
B1	F1	35	15	42.8	12	34.2	8	22.8
B2	F1	35	16	45.7	10	28.5	9	25.7
E	F2	44	15	34.0	16	36.3	13	29.5
F	F2	28	11	39.2	8	28.5	9	32.1
Total		142	57	40.1	46	32.3	39	27.4
Total F1		70	31	44.2	22	31.4	17	24.2
Total F2		72	26	36.1	24	33.3	22	30.5

The most important fact for the present is that conjugation within a clone of circulars results in the production of ellipticals, circulars, and in-

intermediates, and that this result is repeated when an F2 circular clone conjugates. This is unmistakable evidence that the different forms under consideration are inherited through conjugation.

f. Similarity and dissimilarity of the descendants of the two members of a pair of conjugants

In the experiments just described, the two original conjugants were in every case circulars, but after conjugation, as we have seen, the descendants were in some cases circular, in others normal elliptical, in others intermediate—all the descendants of any one ex-conjugant being of the same type. The question arises: are the two lines descended from the two members of the same pair of conjugants always of the same type?

This question is answered in table 2. There were altogether, in the four groups B1, B2, E, and F of figure 2, 46 pairs in which the form could be determined for both members of the pairs. As shown in table 2 the two lines descended from the two members of a pair are not always of the same type, though in many cases they are. Of the 46 pairs, in 34 the descendants of the two members were alike in form while in 12 they were diverse. Table 2 shows for the four groups above mentioned the distribution of the 92 lines derived from the 46 pairs with relation to the three types of form, with the numbers and percentages in each group of the three types of form. On the whole 73.9 percent of the pairs had progeny of like type, while 26.1 percent had descendant lines of unlike types.

TABLE 2

Number and percentage of complete pairs in which the two members were alike and the two were unlike, the distribution of the pairs as to kind of likeness and unlikeness, in each group, and the totals of these for four groups. E= normal elliptical; C= circular; I=intermediate.

GROUP	NUMBER COMPLETE PAIRS	LIKE PAIRS					UNLIKE PAIRS				
		NUMBER	PERCENT	E-E	C-C	I-I	NUMBER	PERCENT	E-I	C-I	E-C
B1	12	6	50.0	4	2	0	6	50.0	4	2	0
B2	10	9	90.0	5	2	2	1	10.0	0	1	0
E	16	13	81.2	6	3	4	3	18.8	1	2	0
F	8	6	75.0	3	0	3	2	25.0	0	1	1
Total	46	34	73.9	18	7	9	12	26.1	5	6	1

Is any general tendency shown toward likeness or toward unlikeness of type between the descendants of the two members of a pair? JENNINGS (1913) and JENNINGS and LASHLEY (1913), working with *Paramecium*, and COHEN (1934) with *Euplotes*, showed that the descendants of the two members of a given pair are on the whole more alike in fission rate than are

lines paired at random. In most of these cases the conjugation was within a single clone. JENNINGS and LASHLEY (1913) and STOCKING (1915) showed a similar relation with respect to death after conjugation and with respect to abnormalities. The two lines descended from the two members of a pair have the same fate more frequently than would be given by random pairing of the ex-conjugant lines. JENNINGS and LASHLEY (1913) consider this greater resemblance in fate to indicate that both the descendant lines are influenced by the contributions from both members of the pair of conjugants; so that they spoke of it as "biparental inheritance."

What is the situation as to this in respect to form in *Euplotes*? This may be determined by comparing the proportions of like and unlike pairs in table 2 with what would be found in case the lines were paired at random. Such a comparison involves the following:

The 46 pairs of table 2 give rise of course to 92 ex-conjugant lines. Of these, as may readily be deduced from the table, 42 are normal elliptical, 21 are circular, and 29 are intermediate. The proportions 42E:21C:29I are almost exactly as 4E:2C:3I. Assuming this latter set of ratios, the results of pairing the lines at random are readily calculated (essentially by squaring 4E:2C:3I). This gives the following proportions:

$$16EE+4CC+9II+16EC+24EI+12CI=81$$

Reducing these proportions to percentages and comparing these calculated percentages to those actually found, we have the relations shown in table 2a.

TABLE 2A

Percentages of diverse types of pairs of lines derived from ex-conjugants, as compared with those calculated from random mating.

CONSTITUTION OF PAIRED LINES	CALCULATED PERCENT	FOUND PERCENT
E E	19.75	39.13
C C	4.94	15.22
I I	11.11	19.57
Total like pairs	35.80	73.92
E I	29.63	10.87
C I	14.81	13.04
E C	19.75	2.18
Total unlike pairs	64.19	26.09

As table 2a shows, in all cases the proportion of pairs in which the two members of a pair are alike is much greater than would be the result from random mating, while the proportion of pairs in which the two are unlike is in every case less than would result from random mating. From random mating 35.8 percent of the pairs should show like members; actually 73.9

percent show like members. From random mating, 64.2 percent would be unlike; actually but 26.1 percent have unlike members.

The evidence is strong therefore that in consequence of conjugation there is a tendency for the descendants of the two members of a given pair to be alike in form. As to the genetic processes to which this is due little that is specific can be said. The general question is discussed in a later section.

Viability

The viability after conjugation shows in these clones of Euplotes certain phenomena that are of interest. The best measure of viability is perhaps the proportion of the ex-conjugant lines that succeed in redifferentiating during the reconstruction stage. Usually, as will be seen, a considerable number of lines fail to redifferentiate and therefore die out. In certain clones all or nearly all thus fail to redifferentiate; this it was that made it seem worth while to bring together the facts on viability after conjugation.

The statistics as to viability for most of the diverse ex-conjugant groups shown in figure 2 are collected in table 3. In this table the groups C, D1, and D2 are omitted, being reserved for special consideration later.

TABLE 3

Number and percentage of lines redifferentiated, of the total number of lines in two P1, two F1, and two F2 groups, showing the relative viabilities.

GROUP.....	A α	A β	B1	B2	E	F
Genetic relationship.....	P1	P1	F1	F1	F2	F2
Total number ex-conjugants.....	174	273	96	144	96	96
Number redifferentiated.....	94	177	61	76	48	43
Percent redifferentiated.....	54.0	64.8	63.6	52.8	50.0	44.8

As can be seen, in the six ex-conjugant groups of table 3 the proportion that redifferentiated after conjugation varied from 44.8 percent to 64.8 percent. Thus the proportion that were inviable was rather high, varying from 35.2 percent up to 55.2 percent.

In the three remaining groups, C, D1, and D2 (figure 2), all of the ex-conjugants were inviable, none of them redifferentiating. In group C, of the 72 conjugating pairs that were isolated, all members of the 144 ex-conjugants were dead 7 days after conjugation, while the non-conjugants continued to live in mass culture.

Group D1

The groups D1 and D2 are of interest from the fact that they were derived from the clone produced by the individual 45a, which individual

arose from the division of one member of a pair of conjugants that never separated. This division took place 11 days after conjugation. This, and the fact that the individual produced was at first weak and did not divide for two days, indicate that the nuclear processes of conjugation had taken place.

In group D1 conjugation occurred on April 1, and as in group C, 72 pairs were isolated. At the same time, as usual, a mass culture of non-conjugants was started; and also in this case a mass culture of conjugating pairs.

Of the conjugants on slides, all but 13 were dead on the day following conjugation (11 single individuals and both members of one pair surviving). To test whether the 60 slide concavities in which ex-conjugants had died might carry injurious conditions, a single non-conjugant was placed in each of these, and this was later repeated as other ex-conjugants died. The non-conjugants flourished, there being but 6 deaths out of the total of 71. It is clear therefore that the death of the ex-conjugants was not due to the slides.

Of the 13 remaining ex-conjugants, all but two were dead the next day (April 3). The two remaining ones later died.

In the mass culture of ex-conjugants, all were dead on April 3 except 4. Some of these four remained alive until April 12, when the last ones died. In the meantime the mass culture of non-conjugants flourished. They were found to be in conjugation on April 9, producing the conjugant group D2 of figure 2.

It is clear from this experiment that the animals were made by conjugation incapable of living in an environment that would have supported them had conjugation not intervened. It seems probable that this was the case also in group C, before described, though here the matter was not so critically tested.

Group D2

The matter was further tested with the group D2. This was derived by conjugation on April 9 from the same clone from which had come D1 (see figure 2). Again 72 conjugant pairs were isolated; also mass cultures were made, one with 100 conjugating pairs, the other with non-conjugants. On the 13th, four days after conjugation, it was found that in both the isolation lines and the ex-conjugant mass culture most of the pairs had not separated. Those that had were still undifferentiated, and most of these were weak. By the 20th all the animals in the mass culture had died, the attached pairs without separating. Among the isolation lines many had died, and by the 23rd only two attached pairs remained alive. By the 26th these had died. Throughout this time the non-conjugant mass culture con-

tinued to live and reproduce, remaining in good condition. Thus the inviability of this clone after conjugation, at least in the environment provided, is well established.

It is therefore clear that in some cases conjugation results in inviability of all the ex-conjugants, and this under conditions in which the non-conjugants flourish. It is of interest that the two groups D1 and D2, in which there was 100 percent inviability, were derived from the same clone, which itself arose from division of a member of a pair in which the two members never separated. This suggests that there was some genetic peculiarity in this clone, giving rise to the total inviability after conjugation. The group C, likewise 100 percent inviable after conjugation, arose however from another individual (see figure 2).

One other fact in connection with these experiments seems worth pointing out. The clone 45a, which arose from a pair of conjugants that died without separating, in a subsequent conjugation (D2) produced conjugants in which this tendency to remain attached again appeared. In the D1 group the lethal effect of conjugation was produced so quickly that there was no opportunity for observing whether the tendency to remain attached occurred here also. But in D2 it was manifested to a great extent, in most of the pairs, and this seems good evidence that the failure of pairs to separate is an inherited tendency.

Fission Rate in Relation to Form

The records on fission rate show that there is a relation between this characteristic and the form of the body; a relation that may perhaps be characterized as linkage. The data on these matters are drawn mainly from the study of group B1 (figure 2), which was examined more fully than the other groups.

In B1 the ex-conjugant lines were followed in detail for 27 days. In table 4 are shown for the three types of form, the number of fissions for three successive nine day periods in each of the 35 lines that redifferentiated after conjugation, together with the mean daily fission rate for each line. (In the case of lines that died out, the fission rate is based on the number of days during which the lines continued to divide.)

Table 4 shows that there was a marked difference between the fission rates in the normal elliptical group and those in the other two groups. In the elliptical group the fission rates are on the whole much higher. This is well shown in the averages for the three forms, given in table 5. The average rate for the ellipticals is double that of the others. The slight difference between the means for the circulars and intermediates (.42 and .47) is hardly significant in view of the small numbers; these two groups have about the same mean fission rate.

TABLE 4

Number of fissions for three 9 day periods, for the total of 27 days, and the mean daily fission rate, of the individual elliptical, circular, and intermediate lines of group B1.

CHARACTERISTIC	LINE	9 DAY PERIOD			TOTAL FISSIONS	NUMBER OF DAYS OF FISSION	MEAN DAILY FISSION RATE
		1	2	3			
Elliptical	2a	5	12	10	27	27	1.00
	4a	6	11	10	27	27	1.00
	5a	6	11	10	27	27	1.00
	5b	6	7	x	13	18	.72
	9b	6	10	11	27	27	1.00
	10a	6	11	10	27	27	1.00
	11a	6	12	10	28	27	1.04
	11b	5	12	11	28	27	1.04
	12b	6	11	11	28	27	1.04
	13a	6	11	11	28	27	1.04
	13b	6	11	11	28	27	1.04
	16a	5	11	10	26	27	.96
	33a	6	11	10	27	27	1.00
	33b	2	x		2	8	.25
	43a	6	11	11	28	27	1.04
Circular	1a	2	x		2	7	.29
	1b	4	8	6	18	31+	.58
	3a	1	x		1	6	.17
	6a	5	5	3	13	27	.48
	7a	2	x		2	7	.29
	7b	5	8	5	18	27	.67
	8b	5	7	1	13	27	.48
	21a	2	x		2	7	.29
	22b	3	4	2	9	27	.33
	30a	2	x		2	7	.29
	34b	4	5	3	12	27	.44
	45a	0	9	9	18	23	.78
Intermediate	2b	4	3	x	7	13	.54
	6b	5	10	10	25	27	.93
	8a	1x			1	6	.17
	9a	3	3	x	6	14	.43
	10b	2	x		2	8	.25
	12a	5	9	4	18	31+	.58
	18a	4	1	x	5	10	.50
	36b	2x			2	6	.33

TABLE 5

Number and percentage of lines viable, and the means of the mean daily fission rates, for the total elliptical, circular, and intermediate lines of group B1, based on the data of table 4.

CHARACTERISTIC	NUMBER OF LINES	NUMBER OF LINES VIABLE	PERCENT VIABLE	MEAN DAILY FISSION RATE
Elliptical	15	13	86.6	.94
Circular	12	4	33.3	.42
Intermediate	8	4	50.0	.47

Within each group there are variations in the fission rate, and some of these appear of much interest. The two low lines (5b and 33b) of the elliptical group are hardly significant, since these lived but a few days. But in the intermediate group one line (6b) has a rate (.93) practically double that of the average for this group, and very near to the average rate for the elliptical group. Also, one of the lines of the circular group (45a) has a rate of .78, while another (34b) that lived throughout the entire period has a rate of but .26, or just one-third that of the high line 45a. These facts indicate that low and high rates may not be indissolubly linked to circular and elliptical forms respectively.

As seen in table 4, the proportion of lines that lived the full 27 days of observation varies in the three form groups. In all the lines which died out before the end of this period there was a distinct "running down," marked by, first, weakness of the animals, then the cessation of division, and finally a more or less protracted wasting away followed by death. Those lines which escaped this running down may be spoken of as "viable" in comparison to the others, although this viability is clearly different from that discussed in the preceding section. Table 5 gives the percentage of such viable lines in the three form groups. As was true for fission rate, the normal elliptical lines have far the highest viability.

DISCUSSION

Conjugation within a clone of protozoa has been regarded, on the basis of the known cytological facts, as showing the essential characteristics of inbreeding, or perhaps more specifically self-fertilization, as the term is used in Mendelian genetics to denote mating between gametes arising vegetatively from the same original fertilized egg. Inbreeding or self-fertilization, however, involves fundamentally the germinal materials that are the basic determining factors in all types of mating: their arrangement and segregation in the parents; their distribution to the progeny; their rearrangement and resegregation in the progeny. In biparentally reproducing metozoa and metaphyta, these things are well understood, and the way in which they occur has been formulated to a degree which permits the co-ordination of most of the observable facts into a closely integrated scheme, and warrants the prediction and verification of additional facts. But in the protozoa they are not so well understood, and it still remains to be proved that inbreeding in these forms actually involves true Mendelian factors.

It is true, however, that many of the facts that have appeared in the study of conjugation in protozoa at least indicate this. In the production of new biotypes by conjugation, for example, the suggested interpretation is that factors which determine the characteristics of the new biotypes become segregated and then recombined in new arrangements during the

formation, exchange, and fusion of pronuclei at conjugation. But in order to prove that such segregation and recombination is of the Mendelian type, it is necessary to study diverse characteristics which are sufficiently contrasted, so that the behavior of the determining factors is sharply reflected in the characteristics, and to obtain them after conjugation in definite ratios that are constant. It is the lack of this type of result that has tended to obscure the essential significance of mating in protozoa, as regards the behavior of genetic factors. Fission rate, viability, and size, although their study has yielded much valuable information, do not present sufficiently contrasted differences or definite enough ratios to permit an examination of the precise behavior of the factors which determine them.

The experimental facts that have been demonstrated in the present account, which is concerned with inbreeding in morphologically distinct clones and with the inheritance of clearly contrasted characteristics, may throw some light on the problem. As pointed out in the introduction, MACDOUGALL has already made a step in this direction. She has shown so far that by conjugation individuals having well defined characteristics of diverse types can arise from a clone in which the parent individuals have these characteristics either combined or not expressed.

In the present case it has been shown that when a clone of circulars derived from normal ellipticals is inbred the progeny may be either elliptical, circular, or intermediate. In Mendelian genetics this is one of the most familiar of the types of results obtained in breeding experiments. It seems probable that the circular parents are heterozygous, in the sense of carrying both dominant and recessive factors that determine form. The simplest type of factor combination that can produce this result by inbreeding is a single pair, represented in each parent by a dominant and a recessive factor. However, the ratio obtained from such a type of mating, apparently one homozygous dominant to two heterozygous to one homozygous recessive, does not at all compare with the ratios obtained from the inbred circulars, so that the possibility of a single pair of factors being involved here is eliminated. Multiple factors are perhaps concerned, although it is not entirely clear how the results observed could be produced by multiple factors.

An examination of the ratios for the different types of progeny, in table 1, shows perhaps sufficient agreement in successive conjugations to indicate a constancy in arrangement and behavior of the genetic factors that determine form. The extreme ratios here for any one type of characteristic, in different conjugations, do not differ by a greater amount than approximately 10 percent. Considering the small number of lines on which each ratio is based, and the extent of the inviability both before and after re-

differentiation that removed so many lines from observation, this degree of constancy seems to have some significance.

In section f, above, it was shown that in most (though not all) cases, the descendants of the two members of a pair of conjugants have the same form. In terms of genetic factors, they inherit like sets of determiners for this characteristic. This possibly indicates something about the nature of the segregation of paired factors in the parents. But before examining the data in this connection, it will be necessary first to consider the known cytological facts.

According to the work of TURNER (1930) the maturation processes of *Euplotes patella* are somewhat different from those of most other protozoa in which they have been studied. In typical maturation in protozoa generally (JENNINGS 1929, pp. 131–135), the micronucleus divides twice, forming four, and during these two divisions reduction occurs. Three of the four then disintegrate and the remaining one divides once, forming the two pronuclei, one stationary and one migratory. The unique effect of this process, as compared with meiosis in higher organisms, is that if reduction really occurs only during the first two divisions, the formation of the two pronuclei or gametes from only one of the four reduced products requires that these two gametes be alike genotypically. The consequence of this is that regardless of how diverse the genotypes of two conjugating individuals are, the two ex-conjugants must become exactly similar in genotype as a result of the exchange of pronuclei. It is apparently the occurrence of this in most cases that JENNINGS had in mind as an explanation of biparental inheritance, that is, the tendency for members of pairs to be more alike than two individuals taken at random that had not mated together.

TURNER, however, finds that in *Euplotes* the maturation phenomena are more complicated. In his account (pp. 212–219), a summary of which is shown diagrammatically in figure 3, it appears that the micronucleus first undergoes a preliminary division and that the first maturation division involves both of these products, forming 4. At the second maturation division, each of the 4 divides and the 8 chromosomes of each become reduced to 4. Of the 8 reduced nuclei, 6 degenerate and the remaining 2 undergo the third maturation division. Of the 4 thus produced, one becomes the stationary pronucleus and one the migratory pronucleus, but it is not possible to tell whether one mother nucleus gives rise to both pronuclei or whether each is derived from a separate mother. So in the one case the two would be alike, in the other they might be different, genotypically.

This uncertainty concerning the immediate origin of the pronuclei prevents making a ready check of the genetic results obtained in the present work by the cytological evidence described by TURNER. It appears, how-

ever, that the genetic evidence may furnish a clue to this doubtful phase of the cytological events.

If the two pronuclei arise from a single mother nucleus (figure 3, first possibility), they must of course have similar genetic factors. However, conjugation between two individuals, each of which has two genotypically similar pronuclei, results in genotypically similar zygote nuclei for the two conjugants, so that by this assumption in all conjugants the two members of a pair should be similar in their inherited characteristics. The genetic

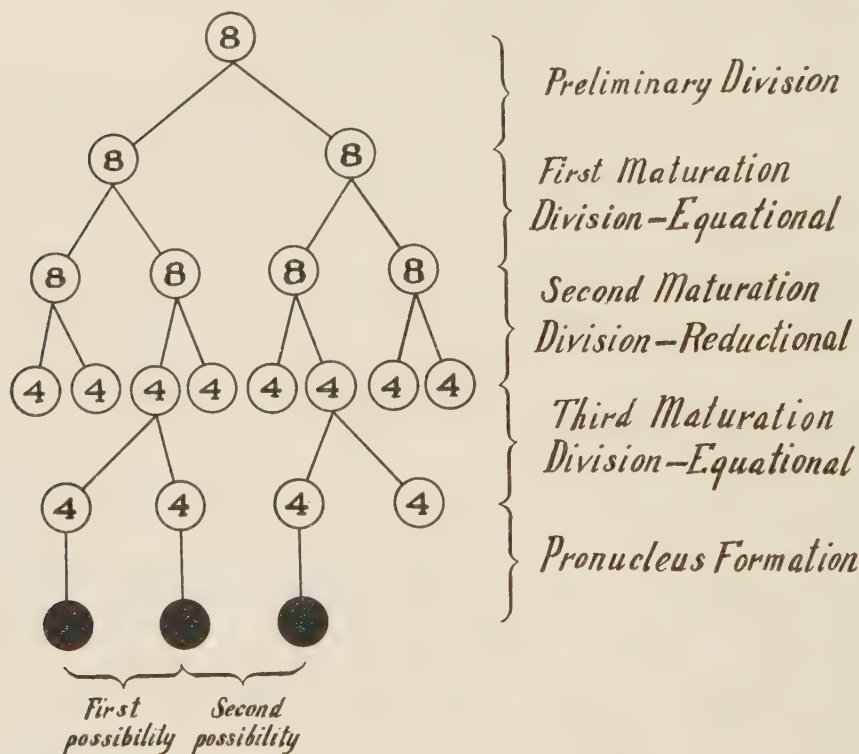


FIGURE 3.—Diagrammatic summary of maturation during conjugation in *Euplotes patella*, based on the account of TURNER (1930). The numerals refer to number of chromosomes.

evidence of the present work, however, shows that in only about 74 percent of the pairs are the two alike in inherited characteristics. The indication is that probably not in every conjugating individual are the two pronuclei formed from the same mother nucleus.

To examine this possibility we may assume that the original micronucleus, before the preliminary division of maturation, is heterozygous for one pair of factors, Aa . Then of the 8 nuclei formed in the reduction division 4 will have the factor A and 4 the factor a . Two of these become the mother nuclei, from one or both of which the gametic pronuclei arise. Since

it is apparently a matter of chance which two of the eight will have this fate, each mother nucleus may have either of the factors, A or a , the chances for either being 50 percent. If the two pronuclei arise from separate mothers (figure 3, second possibility), in their case also each may have either the factor A or the factor a , so that the two pronuclei of any conjugant may be A and A , A and a , a and A , or a and a , the chances for each combination being 25 percent. On the basis of random exchange of pronuclei between two conjugants the expected ratio of like to unlike pairs may be readily calculated and turns out to be 37.5 percent to 62.5 percent. The ratio actually obtained, 74 percent to 26 percent, does not agree with this and so indicates that the two pronuclei do not in every case form from separate mother nuclei.

The genetic evidence, therefore, seems to show that the gametic pronuclei arise sometimes from one mother nucleus, sometimes from two. If in 50 percent of the conjugants they arise from one mother, and in 50 percent from two, the expected ratio of like to unlike pairs is 69 percent to 31 percent which agrees fairly closely with the ratio obtained.

The facts concerning viability cannot all be readily interpreted in terms of Mendelian factors. Low viability may perhaps be due to recessives that are lethal when homozygous. But complete inviability cannot be explained in this way. The significance of this latter phenomenon is in fact quite obscure, since no satisfactory hypothesis of any kind has as yet been advanced for it.

The indications that fission rate and viability are in some way related to the form characteristics in inheritance are of interest. The data may be explained by assuming either that the same factor or factors that determine the circular form also influence fission rate and viability adversely, or that these characteristics are determined by other factors which are distributed with those for circular, and so illustrate linkage of the familiar Mendelian type. The extremely tentative nature of these suggestions, however, warrants no more for the present than their mere statement.

It is perhaps best not to attempt to carry the analysis of the present experimental facts farther than this. The foregoing discussion has been concerned primarily with suggestions for interpretation of these facts, on the assumption that Mendelian factors and Mendelian inheritance are involved in the genetics of Euplotes. It should perhaps be emphasized that this has remained throughout only an assumption, made plausible largely by the fact that inheritance in higher organisms has been successfully worked out with the aid of these concepts. It is, however, by no means clear to what extent they can be applied readily to observations on inheritance in the Protozoa.

There is a great temptation, when confronted with such facts as those

observed in the present work, to take for granted that paired genes, arranged linearly in paired chromosomes, are the determining agents concerned. But genes, of the type known in *Drosophila*, have not been demonstrated in Protozoa. It still remains possible that some entirely non-Mendelian system may be responsible for many of the observations that have been made in Protozoan genetics.

SUMMARY

1. In the course of conjugation in a clone of normal elliptical *Euplotes*, the two members of a certain pair gave origin to two lines of circular individuals.

2. These circular individuals when inbred gave origin by conjugation to normal elliptical, circular, and intermediate progeny.

3. These characteristics appeared in four different conjugations of the circulars in approximately the same ratios (averaging about 40 ellipticals to 32 circulars to 28 intermediates).

4. The two lines descended from the two members of the same pair of conjugants may have the same form (EE, CC, or II), or may differ in form (EC, EI, or CI). (E=elliptical, C=circular, I=intermediate.)

5. The proportion of cases in which the two lines from the same pair were alike (about 74 percent of all) was much above what would be produced by random pairing of the different lines resulting from conjugation.

6. The viability after conjugation was low, varying from about 65 percent to 0. This low viability ran through three successive conjugations. In one clone (45a) the viability was 0 in two different conjugations, indicating a genetic peculiarity of this clone.

7. Among the clones of different form produced by inbreeding in a circular clone, the normal elliptical clones were considerably higher in both viability and fission rate than the circular and intermediate clones. The two latter differed little in these respects.

8. The relation of the above facts to the known cytological processes and to Mendelian inheritance is discussed.

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